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Evaluating the Similarity of Complex Drinking-Water Disinfection By-Product Mixtures: Overview of the Issues

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Humans are exposed daily to complex mixtures of environmental chemical contaminants, which arise as releases from sources such as engineering procedures, degradation processes, and emissions from mobile or stationary sources. When dose-response data are available for the actual environmental mixture to which individuals are exposed (i.e., the mixture of concern), these data provide the best information for dose-response assessment of the mixture. When suitable data on the mixture itself are not available, surrogate data might be used from a sufficiently similar mixture or a group of similar mixtures. Consequently, the determination of whether the mixture of concern is "sufficiently similar" to a tested mixture or a group of tested mixtures is central to the use of whole mixture methods. This article provides an overview for a series of companion articles whose purpose is to develop a set of biostatistical, chemical, and toxicological criteria and approaches for evaluating the similarity of drinking-water disinfection by-product (DBPs) complex mixtures. Together, the five articles in this series serve as a case study whose techniques will be relevant to assessing similarity for other classes of complex mixtures of environmental chemicals. Schenck et al. (2009) describe the chemistry and mutagenicity of a set of DBP mixtures concentrated from five different drinking-water treatment plants. Bull et al. (2009a, 2009b) describe how the variables that impact the formation of DBP affect the chemical composition and, subsequently, the expected toxicity of the mixture. Feder et al. (2009a, 2009b) evaluate the similarity of DBP mixture concentrates by applying two biostatistical approaches, principal components analysis, and a nonparametric "bootstrap" analysis. Important factors for determining sufficient similarity of DBP mixtures found in this research include disinfectant used; source water characteristics, including the concentrations of bromide and total organic carbon; concentrations and proportions of individual DBPs with known toxicity data on the same endpoint; magnitude of the unidentified fraction of total organic halides; similar toxicity outcomes for whole mixture testing (e.g., mutagenicity); and summary chemical measures such as total trihalomethanes, total haloacetic acids, total haloacetonitriles, and the levels of bromide incorporation in the DBP classes.

Humans are exposed daily to complex mixtures of environmental contaminants. The U.S. Environmental Protection Agency (U.S. EPA, 2000) defines a complex mixture as a mixture containing so many components that any estimation of its toxicity based on its components' toxicities contains too much uncertainty and error to be useful. The chemical composition may vary over time or with different conditions under which the mixture is produced. Complex mixture components may be generated simultaneously as by-products from a single source or process, intentionally produced as a commercial product, or may coexist because of disposal practices. Risk assessments of complex mixtures are preferably based on toxicity and exposure data on the complete mixture.

Complex mixture exposures arise as emissions from mobile (e.g., automobile exhaust) or stationary sources (e.g., industrial emissions, waste incinerators), engineering procedures (e.g., chemical disinfection of drinking waters), and degradation processes (e.g., chemical transformation of applied pesticides through weathering). Uses of manufactured products, such as pesticides, fuels, and polybrominated diphenyl ethers (PBDE) that are in flame retardants, also result in human exposures to complex mixtures. Many environmental mixture exposures occur inadvertently; contacts with urban and household air,

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workplace dusts, Superfund site emissions, and mine tailings all result in human exposures to complex mixtures that are coincident in time and place (DeRosa et al., 2004).. These contaminants co-occur because the properties governing their environmental fate are similar. Given the wide array of environmental mixtures, evaluating the risks they pose to human health is challenging, yet important.

The U.S. EPA issued both guidelines (U.S. EPA, 1986) and general guidance (U.S. EPA, 2000) that discuss methods for assessing health risks associated with exposures to chemical mixtures. Two categories of methods are proposed based on data availability, those based on whole mixtures and those based on the mixtures' components (U.S. EPA, 2000). If whole-mixture toxicity data are available, they are preferred in environmental risk assessments. The alternative is to apply methods that use individual chemical component data only (e.g., dose addition, response addition), which are based on simplifying assumptions and are associated with more uncertainty than whole mixture approaches. Figure 1 illustrates the choice of risk assessment methods for whole mixtures (third row), which depends on knowledge of the chemical composition and toxicity of a complex mixture or of other mixtures that might be considered similar to it. (For a quick reference regarding how whole mixtures methods may be applied, see the User-Fact Sheets in Section 2.5 of U.S. EPA [2000].[1])

When dose-response data are available for the whole mixture to which individuals are exposed (i.e., the mixture of concern), the U.S. EPA (2000) recommends the use of these data in the risk assessment, because they should provide the best information for developing an accurate estimate of the dose-response relationship for the mixture. When dose-response data are not available for the mixture of concern, the U.S. EPA (2000) recommends the use of dose-response data from a similar mixture (i.e., the tested mixture) or from a group of similar mixtures. Such mixtures would be expected to cause similar toxicological effects and exhibit similar effect magnitudes (e.g. similar slope factors). Consequently, the determination of whether the mixture of concern is "sufficiently similar" to a tested mixture or a group of tested mixtures is central to the use of whole mixture methods.

The assessment of health risks associated with whole mixtures is challenging because typically these complex mixtures are comprised of many components that vary depending on how the mixture was produced; environmental factors that may act to alter differentially the mixture composition; and where and how humans come into contact with the mixture. Because the chemical composition of such mixtures may vary over time

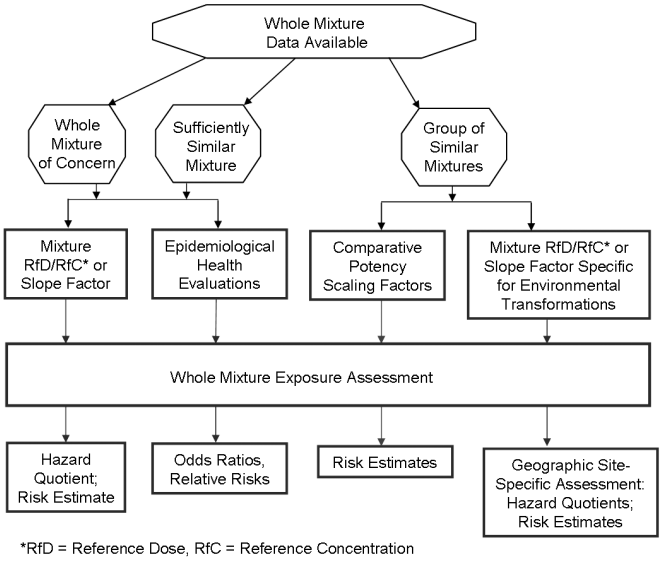

FIG. 1. Flow chart of complex chemical mixtures risk assessment methods.

or with different conditions under which the mixture is produced, understanding the likelihood and nature of potential health risks posed by exposure to such similar mixtures is critical. It is recognized that both the magnitudes and the types of the health risks associated with an environmental mixture might change with variations in exposure scenarios and with changes in the composition of the mixture.

This article provides an overview for a series of companion articles (Schenck et al., 2009; Bull et al, 2009a, 2009b; Feder et al., 2009a, 2009b) whose purpose was to develop a set of biostatistical, chemical, and toxicological criteria and approaches for evaluating the similarity of complex mixtures of drinking-water disinfection by-products (DBPs). Together, these articles serve as a case study whose techniques will be relevant to assessing similarity for other classes of complex mixtures of environmental chemicals. Schenck et al. (2009) describes the composition and mutagenicity of a set of DBP mixtures concentrated from five different drinking-water treatment plants providing data that are used in the subsequent articles in this series. Bull et al. (2009a, 2009b) describe how the variables that affect the formation of chlorination DBPs affect the chemical composition of the mixture and subsequently, the expected toxicity of the mixture. Feder et al. (2009a, 2009b) evaluate the similarity of DBP mixture concentrates by applying two biostatistical approaches, principal components analysis and a nonparametric "bootstrap" analysis to the chemistry and mutagenticity data in Schenck et al. (2009), along with another chemical data set on 35 water utilities (U.S. EPA/AMWA, 1989). While the toxicity information provided through *in vitro* mutagenicity tests is less relevant to human health risks than *in vivo* toxicology or epidemiology studies, *in vitro* toxicity data are easily obtained, useful for demonstrating

[1]U.S. EPA (2000) presents detailed descriptions of available mixture risk assessment methods, as well as user fact-sheets which provide concise overviews. These method-specific fact-sheets provide information relative to the mixture risk assessment approaches including: the type of assessment (e.g., dose-response assessment, risk characterization), data requirements, references, strategy of the method with calculations, ease of use, assumptions, limitations, and uncertainties.

the statistical approaches, and examine the toxicity of the whole mixture, including the unidentified fraction of the complex DBP mixture.

RISK ASSESSMENT APPROACHES TO SIMILAR MIXTURES

The U.S. EPA (2000) provides limited guidance for evaluating similarity among complex mixtures. If available for the mixtures in question, a minimal analysis is to evaluate similarities in the main components and the component proportions between mixtures. Such data may be supplemented by using environmental fate data for the different mixtures to evaluate their similarity. Finally, consideration needs to be given to the uncertainties associated with using dose-response data on the tested mixture as a surrogate for the environmental mixture and the uncertainties associated with alternative approaches, such as component methods. To date, similarity-based approaches rely on the judgment of the risk analyst. Specific guidance has yet to be developed on transparent and objective methods that could be used to evaluate similarity between mixtures.

Figure 2 illustrates a schematic for estimating risk associated with a mixture of concern based on the toxicity information for the tested mixture. This Figure shows that the key decision is whether the mixture of concern and the tested mixture are similar. If the mixtures are judged to be similar, then the dose-response estimates derived from the toxicity data on the tested mixture (e.g., the reference dose, reference concentration, slope factor[2]) are used as surrogates for estimating the risk of the mixture of concern (see Section 3.0 of U.S. EPA [2000] for details of this procedure). If the mixture of concern and the tested mixture are not similar, then a determination is made pertaining to the feasibility of developing dose-response data for the mixture of concern. The feasibility analysis could include technical and financial (cost of experimentation) considerations. If feasible, then dose-response data may be developed for the mixture of concern and used in its assessment. If it is not feasible to develop dose-response data for the mixture of concern, then component methods needs to be considered to characterize the risk posed by the mixture of concern. Typically, there is less confidence associated with the use of component methods. The types of uncertainties associated with the use of component data may differ substantially from those associated with the uses of whole mixtures methods.

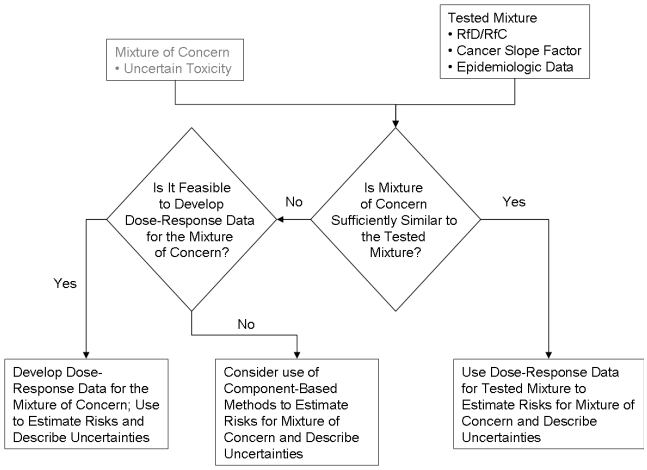

FIG. 2. Schematic for estimating risk associated with the mixture of concern using a sufficiently similar mixture.

These require careful evaluation and, when possible, the risk estimates developed using component methods need to be compared to those of the tested whole mixture.

In making the judgment regarding similarity, the compositions of the mixture of concern and the tested mixture are examined. The mixtures may consist of the same components but exhibit differing ratios, the mixtures may have some unique components, or both. Similarity of dose response among a common group of components for these mixtures can also be evaluated using statistical procedures to identify those components that can be characterized with the same dose-response slope (Chen et al., 2003). Sufficient similarity in dose response has also been defined by using statistical equivalence testing for mixtures of many chemicals containing the same components with different ratios (Stork et al., 2008). Furthermore, it is not unusual for a large portion of complex mixtures to be chemically uncharacterized, resulting in an unidentified fraction whose contribution to toxicity may be unknown but needs to be considered when evaluating similarity. When judging the sufficient similarity of the two mixtures, a goal is to understand how the differences between the mixture of concern and the tested mixture might produce differences in the toxicities of the two mixtures and to what degree such differences might be expressed. This necessitates some understanding of the influence of the components and the unidentified fractions on the toxicities of the mixtures being compared. The results of chemical and toxicological data evaluations and statistical analyses can be used to form criteria for evaluating similarities among complex mixtures.

While analyses of component data are important, "summary measures" that represent and integrate either the toxicity and chemistry (or both) of the complex mixtures provide information to evaluate similarity, including the unidentified fraction: (1) *In vitro* data (e.g., from mutagenticity or cytotoxicity studies), (2)

[2]Reference dose (RfD): an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Reference concentration (RfC): an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Slope factor: an upper bound, approximating a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent.

in vivo toxicological data where animals are exposed directly to the complex mixture or concentrates of the mixture, and (3) epidemiological results offer insights into whole mixture toxicity. Chemical composition can be evaluated using gas chromatography–mass spectrometry (GC-MS) chromatograms, other analytical methods, and summary measures of chemical characteristics of a mixture (e.g., for DBPs important factors might include pH level, percent total organic bromine relative to total organic halide, and others). An example of an integrated protocol is found in Cizmas et al. (2004) in which sufficient similarity was evaluated using *in vitro* bioassays (*Salmonella*/microsome, *Escherichia coli* prophage induction, chick embryo toxicity screening assays) and chemical analysis (GC-MS) for fractionated mixtures of wood preserving wastes containing polycyclic aromatic hydrocarbons (PAH) and pentachlorophenol (PCP). Although these particular mixtures appeared chemically similar, the biological responses they elicited were dissimilar. In addition, Eide et al. (2002) developed a method to predict the mutagenicity of sufficiently similar mixtures of organic extracts of exhaust particles using a combination of pattern recognition techniques and multivariate regression modeling. They constructed a predictive mutagenicity model by regressing the peaks observed in GC-MS chromatograms of these mixtures on the mixtures' mutagenicity measures. Thus, the mutagenicity of a new mixture of exhaust particles was estimated by the model using input from its GC-MS chromatogram.

Figure 3 highlights the use of sufficient similarity for a group of similar mixtures. In the approach, the environmental mixture of concern is compared with a group of tested mixtures using some identified and common characteristics. For example, all of the mixtures may be produced by the same water treatment process (e.g., chlorination), and within those mixtures all may contain a specified set of DBPs whose concentrations fall within an identified range. If the mixture of concern also shares those common characteristics, then toxicity information derived from data on the group may be used to evaluate the mixture of concern. If the mixture of concern and the tested mixtures are not similar, then a determination is made pertaining to the feasibility of developing dose-response data for the mixture of concern. If this becomes a research priority, then ultimately its toxicity data would be added to the library of information on tested mixtures for future reference in risk assessment.

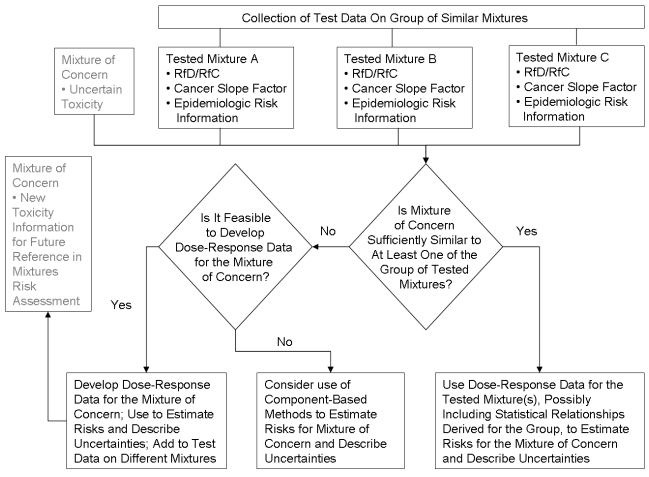

FIG. 3. Schematic for estimating risk associated with the mixture of concern using test data on a group of similar mixtures.

Statistical relationships among a group of similar mixtures may be used to estimate toxicity. The comparative potency approach (Figure 1) is an example where it is assumed that the mixture of concern can be considered a member of a class of similar mixtures (i.e., diesel emissions from different engines) based on similarity of biologic activity, or reasonable expectation of a type of biologic activity based on chemical composition (Lewtas, 1985, 1988; Nesnow, 1990). It was assumed that a uniform proportionality constant exists between assays for all mixtures in the similarity class and for the series of bioassays under consideration. In this procedure, toxicity data from the group of similar mixtures are used to estimate a scaling factor that relates toxic potency between two different assays of the same toxic endpoint. The mixture of concern is then tested in one of the assays (perhaps an inexpensive simple assay, e.g., *in vitro* mutagenicity), and the resulting potency is then adjusted by scaling factors to estimate human cancer potency. In general, for such a procedure, regression models can be used to estimate the scaling factor.

Cogliano (1998) provides an example of how such an approach could work using commercial polychlorinated biphenyl (PCB) carcinogenicity data (i.e., a group of tested mixtures which exhibit different cancer potencies) to estimate the carcinogenicity of environmental PCBs (i.e., mixtures of concern). (In Figure 1 this method is designated as "Geographic Site-Specific Assessments.") The composition of the commercial PCB mixtures (i.e., four different Aroclors) is better understood than the composition of the environmental PCB mixture of concern. Environmental processes alter PCB mixtures through differential partitioning, transformation, and bioaccumulation; these processes can alter the mixture's carcinogenicity. The Cogliano (1998) method considers the effect of environmental processes that result in exposures to the mixture of PCB and then, based on the chemical characteristics of the tested commercial PCB mixtures, assigns a surrogate mixture for use in assessing the carcinogenicity of the environmental PCB mixture. Cogliano (1998) assumes that the composition of the environmental PCB mixture to which subjects are exposed remains reasonably stable over time. Using this assumption, he recommends characterizing the cancer risk using the chronic data from the Aroclor bioassays.

SUFFICIENT SIMILARITY OF COMPLEX DBP MIXTURES

Judgments of sufficient similarity can be extremely challenging for complex mixtures such as drinking-water DBPs.

The chemical disinfection of drinking water produces hundreds of DBPs through reaction of the disinfecting agent (e.g., chlorine, ozone) with the organic matter in the water. Health effects were observed in both toxicological and epidemiological studies (Teuschler & Simmons, 2003; Rice et al, 2008; Simmons & Teuschler, in press). Toxicological studies showed that some individual DBPs are capable of producing adverse effects, including cancer, developmental, reproductive and other noncancer effects, and epidemiological studies associated disinfected water or certain DBPs or classes of DBPs with bladder, colon, and rectal cancers and adverse developmental and reproductive outcomes. Because of the widespread human exposure to mixtures of DBPs, the U.S. EPA is evaluating the potential risks they pose to public health (Simmons et al., 2002, 2008; Rice et al., 2008).

Figure 4 provides a simple illustration of some of the information generally known about how DBP exposures vary with different source water/disinfectant combinations and what associations there are between different types of DBPs and various health effects data from either epidemiological or toxicological studies. For example, high levels of total organic carbon (TOC) in source waters treated with chlorine produce more chlorinated than brominated DBPs and are associated with bladder cancer in epidemiologic studies. In contrast, high levels of bromide in source waters treated with chlorine produce more brominated than chlorinated DBPs; brominated DBPs, in particular bromodichloromethane, are associated with reproductive and developmental effects (Waller et al., 1998). Thus, from a perspective of determining sufficient similarity, an important factor to consider is whether the relative amount of brominated versus chlorinated DBPs is about the same in comparing two finished drinking-water DBP mixtures. Two additional factors that would determine DBP mixtures similarity are similar levels of TOC or bromide in the source water and same type of chemical disinfection. Figure 4 illustrates that

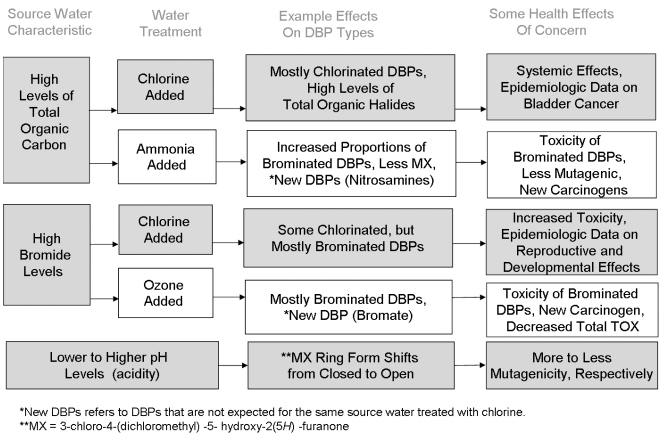

FIG. 4. Examples of known information on the interrelationships among source water characteristics, disinfectant interactions, and health effects data.

if ammonia or ozone are used instead of chlorine for the source waters shown, then different DBPs are formed (i.e., nitrosamines and bromate, respectively) that may change potential cancer risks. Other important water chemistry factors from a toxicological perspective include similar concentrations and proportions of the regulated individual DBPs within the mixtures and a similar unidentified fraction of total organic halide material.

Toxicological factors can also be identified and used to determine similarity among DBP mixtures. Such factors may include similar toxicity outcomes for whole mixture testing, such as mutagenicity measures and similar toxicity for the major chemical components that are common between mixtures. Table 1 illustrates a number of toxicological factors that may be useful in similarity evaluations, including measures of toxicological consistency for dose response and type of health impact and consideration of changes in toxic potency or mode of action. In this series, the Schenck et al. (2009) article shows mutagenicity and DBP chemistry data for 5 water treatment plants; these data are used, along with another data set on 35 water utilities (U.S. EPA/AMWA, 1989) to explore identification of important toxicological and chemical similarity factors. Bull et al. (2009a, 2009b) describe chemical and toxicological criteria for sufficient similarity of DBP mixtures, such as those illustrated in Figure 4 and Table 1, but in much greater detail.

In addition to the development of toxicological and chemical information regarding similarity, statistical approaches are needed to identify similarity factors that are informative and can be used to quantitatively analyze for similarity between mixtures. For example, for two water treatment plants, suppose there are measurements of the concentrations and proportions of individual DBP, on the magnitude of the unidentified fraction of total organic halides, and also on the mutagenic potency of each mixture. Then, graphics and statistical methods can be used to quantitatively determine how different such measures need to be before the two complex mixtures would be declared not sufficiently similar for use in a risk assessment. In this series, Feder et al. (2009a) conducts such an analysis of the five water treatment plants discussed in the Schenck et al. (2009) article using a variety of multivariate graphical and analytical statistical methods including principal components analysis. In addition, Feder et al. (2009b) describes a nonparametric bootstrap method for analyzing sufficient similarity and compares the results to parametric methods. Important summary measures for sufficient similarity that are identified as useful in statistical modeling include mutagenicity data, TOC, total organic halides, total trihalomethanes, total haloacetic acids, total haloacetonitriles, and percentage of such chemical measures that is comprised of brominated compounds.

The principal components analysis method used by Feder et al. (2009a) is a multivariate data transform often used to reduce multidimensional data sets to lower dimensions for analysis. A transformed set of variables is produced that is

TABLE 1
Plausible Toxicological Factors for Evaluating Similarity Across DBP Complex Mixtures

Category	Plausible toxicological factors for evaluating similarity across complex DBP mixtures
Mutagenicity	Are mutagenic responses phenotypically similar? Are the potencies of the mutagenic activities similar?
Health effects	Do the mixtures have the same target organ(s)?
	Is there consistency in the nature of health effects? Are the types or sites of cancer the same?
	Are the health effects consistent with what is known about the chemical composition of the mixtures?
Dose-response	Are the dose-response slopes or shapes similar?
	Is the toxic potency of the mixtures similar?
	For the same endpoint, are the mixtures consistent in the presence/absence of effect thresholds?
	Do the mixtures have similar dose dependence of effects?
	Is there consistency across mixtures of toxic potency for the same effect?
	Is there consistency across mixtures in effects for specific routes of exposure?
Whole mixture effects	Is there consistency of effects between whole mixtures, including the unidentified fraction?
	Is there consistency of health effects between groups of known DBPs and the whole DBP mixtures?
Toxicological interactions	Do the mixtures have a similar potential for toxicological interactions?
	Is the evidence of initiators/promoters consistent across mixtures?
	Is the potential toxicity of metabolic intermediates similar across mixtures?
Mode of action	Is the mode of action the same for groups of chemicals in the mixtures?
	For the same endpoint, is either low-dose linearity or threshold of effects consistent across the mixtures?
Epidemiology	Are epidemiological findings similar across the mixtures?
	Do the mixtures share consistency of toxicological and epidemiological effects?
	Is the potential of effects in sensitive individuals similar across the mixtures?

comprised of statistically independent linear combinations of the original dependent variables. These new variables, called principal component one, principal component two, etc., are those independent directions in the multivariate space that explain as much variation in the data as possible, over and above the variation explained by the lower numbered principal components, with the most variation explained by principal component one, the second most explained by principal component two, etc. The principal component directions are selected based on their ability to explain variation in data but often they reflect physical characteristics of the data, e.g., the difference between groundwater sources and surface water sources.

A hypothetical example of the graphical results that can be obtained from such a principal components analysis is shown in Figure 5, where the plotting symbols C and O correspond to chlorine and ozone disinfection, respectively, G and S correspond to ground and surface water, respectively, and 1, 2, 3, 4, and 5 correspond to the five water treatment plants. In Figure 5, there are three samples represented for each treatment plant with a fixed disinfection scenario and water source. Principal components 1 and 2 are the two statistically independent linear combinations of the responses that explain the most variation

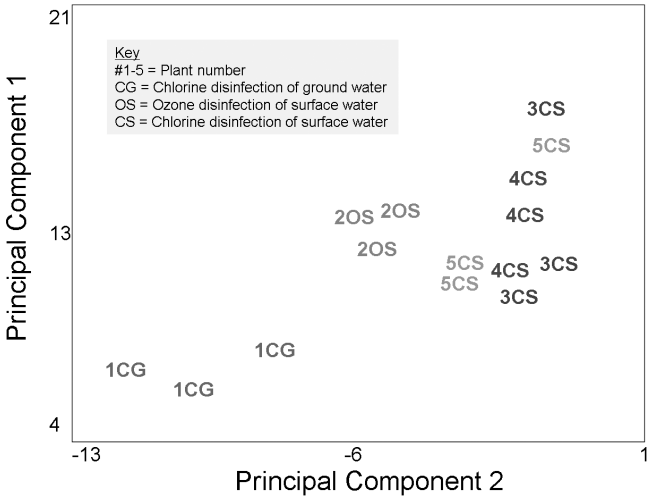

FIG. 5. Hypothetical example of graphical results from a principle component analysis. Principal components 1 and 2 are statistically independent linear combinations of the original dependent variables in the model (e.g., mutagencity, total trihalomethanes). Points that cluster together within the graph are close together in higher dimensional space and so can be interpreted as being "similar."

in the data. When these data points are plotted against values of these first two principal components (Figure 5), much of the higher dimensional variation among the data points is represented in two dimensions. Points that cluster together within the graph are close together in higher dimensional space and so can be interpreted as being "similar." In Figure 5, it is apparent from examining the clusters represented by plants 1–5 in this (hypothetical) graph that the statistical modeling detected differences in the complex DBP mixtures produced by different disinfectants (i.e., the single ozone plant 2 is in its own cluster) and source waters (i.e., the single groundwater plant 1 is in its own cluster).

CONCLUSIONS

Ultimately, the development of similarity approaches for a class of complex mixtures will allow a specified mixture of concern to be characterized as sufficiently similar, or not, to a tested mixture based on comparisons of relatively inexpensive and quick measures between the mixture of concern and the tested mixture. The approaches should yield accurate and reproducible results that can be used to justify scientifically and objectively the use of toxicity information from a similar mixture. An example of this for DBPs would be to produce a library of toxicity and chemistry data on DBP mixtures formed for a representative set of specific disinfectants (e.g., chlorination, ozonation, chloramination), each combined with defined source water characteristics (e.g., low or high levels of bromide or of total organic carbon). This representative library of DBP mixtures information could then be used to evaluate environmental mixtures of DBPs formed from similar source waters and treatment processes (Teuschler & Simmons, 2003; Rice et al., 2008). The ideal approach also would determine when no mixture is found within a group of tested mixtures that can adequately describe the toxicity of a mixture of concern. For a complex class of mixtures such as the DBPs, the development of such a set of tested mixtures is likely many years away; however, the development of approaches for examining the similarity of such mixtures can help to identify (1) the types of toxicity tests, (2) the types of summary measures that need to be developed, and (3) the types of DBP mixtures that need to be examined.

Computational toxicology and "high-throughput toxicology" approaches may offer insights into the similarity of complex mixtures in the near future. When compared with *in vivo* toxicity data, analytic chemistry data require fewer resources to obtain and thus are more readily available. Computational toxicology and "high-throughput toxicology" may offer similar advantages. Evaluations of the transcriptional and translational events associated with the same doses of different (but possibly similar) complex mixtures may provide important insights into the similarities or differences in the toxicity associated with these mixtures. These differences might be related to the differences in the composition or proportions of the known chemicals contained in the mixtures; for example a limited number of DBPs are routinely measured. A longer term goal might be the conduct of analyses of the toxicity pathways associated with different mixtures. Such analyses might provide important insights into differences and similarities among mixtures.

The goal of this series of research articles is to further the development of methods for evaluating the similarity of complex chemical mixtures. While the current application is to DBPs, the ultimate goal is to produce methods that can be generalized and applied to evaluate the toxicity of other complex environmental mixtures. Thus, the approaches and ideas illustrated here with complex mixtures of DBPs will eventually become options in a risk assessment toolkit for evaluations of similarity among mixtures.

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